DETECTION OF CONFORMATIONALLY ALTERED PROTEINS AND PRIONS

RELATED APPLICATIONS

This application is a continuation-in-part application of United States Patent Application Serial No. 10/161,061, filed May 30, 2002, which claims priority from United States Provisional Patent Application Serial No. 60/295,456, filed May 31, 2001.

FIELD OF THE INVENTION

The invention provides methods and kits for detecting conformationally altered proteins and prions in a sample.

In one embodiment, the conformationally altered proteins and prions are associated with amyloidogenic diseases.

BACKGROUND OF THE INVENTION

1. Conformationally Altered Proteins and Prions and Associated Diseases.

The conversion of normally soluble proteins into conformationally altered insoluble proteins is thought to be a causative process in a variety of other diseases. Structural conformational changes are required for the conversion of a normally soluble and functional protein into a defined, insoluble state. Examples of such insoluble proteins include: A. beta. peptide in amyloid plaques of Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA); .alpha.-synuclein deposits in Lewy bodies of Parkinson's disease, tau in neurofibrillary tangles in frontal temporal dementia and Pick's disease; superoxide dismutase in amylotrophic lateral sclerosis; huntingtin in Huntington's disease; and prions in Creutzfeldt-Jakob disease (CJD): (for reviews, see Glenner et al. (1989) J. Neurol. Sci. 94:1-28; Haan et al. (1990) Clin. Neurol. Neurosurg. 92(4):305-310).

Often these highly insoluble proteins form aggregates composed of nonbranching fibrils with the common characteristic of a beta.-pleated sheet conformation. In the CNS, amyloid can be present in cerebral and meningeal blood vessels (cerebrovascular deposits) and in brain parenchyma (plaques). Neuropathological studies in human and animal models indicate that cells proximal to amyloid deposits are disturbed in their normal functions (Mandybur (1989) Acta Neuropathol. 78:329-331; Kawai et al. (1993) Brain Res. 623:142-6; Martin et al. (1994) Am. J. Pathol. 145:1348-1381; Kalaria et al. (1995) Neuroreport 6:477-80; Masliah et al. (1996) J. Neurosci. 16:5795-5811). Other studies additionally indicate that amyloid fibrils may actually initiate neurodegeneration (Lendon et al. (1997) J. Am. Med. Assoc. 277:825-31; Yankner (1996) Nat. Med. 2:850-2; Selkoe (1996) J. Biol. Chem. 271:18295-8; Hardy (1997) Trends Neurosci. 20:154-9).

In both AD and CAA, the main amyloid component is the amyloid beta protein (A. beta.). The A. beta. peptide, which is generated from the amyloid beta precursor protein (APP) by the action of two putative secretases, is present at low levels in the normal CNS and blood. Two major variants, A.beta $_{1-40}$ and A.beta $_{1-42}$, are produced by alternative carboxy-terminal truncation of APP (Selkoe et al.(1988) Proc. Natl. Acad. Sci. USA 85:7341-7345; Selkoe, (1993) Trends Neurosci 16:403-409). A.beta $_{1-42}$ is the more fibrillogenic and more abundant of the two peptides in amyloid deposits of both AD and CAA. In addition to the amyloid deposits in AD cases described above, most AD cases are also associated with amyloid deposition in the vascular walls (Hardy (1997), supra; Haan et al. (1990), supra; Terry et al., supra; Vinters (1987), supra; Itoh et al. (1993), supra; Yamada et al. (1993), supra; Greenberg et al. (1993), supra; Levy et al. (1990), supra). These vascular lesions are the hallmark of CAA, which can exist in the absence of AD.

Human transthyretin (TTR) is a normal plasma protein composed of four identical, predominantly beta.-sheet structured units, and serves as a transporter of the hormone thyroxin. Abnormal self assembly of TTR into amyloid fibrils causes two forms of human diseases, namely senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP) (Kelly (1996) Curr Opin Struct Biol 6(1):11-7). The cause of

amyloid formation in FAP is point mutations in the TTR gene; the cause of SSA is unknown. The clinical diagnosis is established histologically by detecting deposits of amyloid in situ in biopsy material.

To date, little is known about the mechanism of TTR conversion into amyloid *in vivo*. However, several laboratories have demonstrated that amyloid conversion may be simulated *in vitro* by partial denaturation of normal human TTR [McCutchen, Colon et al. (1993) Biochemistry 32(45):12119-27; McCutchen and Kelly (1993) Biochem Biophys Res Commun 197(2) 415-21]. The mechanism of conformational transition involves a monomeric conformational intermediate which poly_merizes into linear beta.-sheet structured amyloid fibrils [Lai, Colon et al. (1996) Biochemistry 35(20):6470-82]. The process can be mitigated by binding with stabilizing molecules such as thyroxin or triiodophenol (Miroy, Lai et al. (1996) Proc Natl Acad Sci USA 93(26):15051-6).

The precise mechanisms by which neuritic plaques are formed and the relationship of plaque formation to the disease-associated neurodegenerative processes are not well-defined. The amyloid fibrils in the brains of Alzheimer's and prion disease patients are known to result in the inflammatory activation of certain cells. For example, primary microglial cultures and the THP-1 monocytic cell line are stimulated by fibrillar .beta.-amyloid and prion peptides to activate identical tyrosine kinase-dependent inflammatory signal transduction cascades. The signaling response elicited by .beta.-amyloid and prion fibrils leads to the production of neurotoxic products, which are in part responsible for the neurodegeneration. C. K. Combs et al, J Neurosci 19:928-39 (1999).

2. Prions.

Prions are infectious pathogens that cause central nervous system spongiform encephalopathies in humans and animals. Prions are distinct from bacteria, viruses and viroids. A potential prion precursor is a protein referred to as PrP 27-30, a 28 kdalton

hydrophobic glycoprotein that poly_merizes (aggregates) into rod-like filaments found as plaques in infected brains. The normal protein homologue differs from prions in that it is readily degradable, whereas prions are highly resistant to proteases. It has been suggested that prions may contain extremely small amounts of highly infectious nucleic acid, undetectable by conventional assay methods Benjamin Lewin, *Genes IV* (Oxford Univ. Press, New York, 1990 at p.1080. The predominant hypothesis at present is that no nucleic acid component is necessary for the infectivity of prion protein.

Complete prion protein-encoding genes have since been cloned, sequenced and expressed in transgenic animals. PrP^{C} is encoded by a single-copy host gene and is normally found at the outer surface of neurons. During a post-translational process, PrP^{Sc} is formed from the normal, cellular PrP isoform (PrP^{C}), and prion diseases result from conversion of PrP^{C} into a modified isoform called PrP^{Sc}. PrP^{Sc} is necessary for both the transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans.

The normal prion protein (PrP) is a cell-surface metallo-glycoprotein that is mostly an alpha-helix and coiled-loop structure as shown in Figure 8, and is usually expressed in the central nervous and lymph systems. It is believed to serve as an antioxidant and is thought to be associated with cellular homeostasis. The abnormal form of PrP, however, is a confor_mer which is resistant to proteases and is predominantly beta-sheet in its secondary structure, as shown in Figure 9. It is believed that this conformational change in secondary structure leads to aggregation and eventual neurotoxic plaque deposition in the prion-disease process.

Prion-associated diseases include scrapie of sheep and goats, chronic wasting disease of deer and elk, and bovine spongiform encephalopathy (BSE) of cattle (Wilesmith, J. and Wells, Microbiol. Immunol. 172:21-38 (1991)). Four prion diseases of humans have been identified: (1) kuru, (2) Creutzfeldt-Jakob disease (CJD), (3) Gerstmann-Strassler-Scheinker Disease (GSS), and (4) fatal familial insomnia (FFI) (Gajdusek, D. C., Science 197:943-960 (1977); Medori et al., N. Engl. J Med. 326:444-

449 (1992)).

Prion diseases are transmissible and insidious. For example, the long incubation times associated with prion diseases will not reveal the full extent of iatrogenic CJD for decades in thousands of people treated with cadaver-sourced HGH worldwide. The importance of detecting prions in biological products has been heightened by the possibility that bovine prions have been transmitted to humans who developed new variant Creutzfeldt-Jakob disease (nvCJD) (G. Chazot et al., Lancet 347:1181 (1996); R. G. Will et al. Lancet 347:921-925 (1996)).

Diseases caused by prions are hard to diagnose: the disease may be latent or subclinical (abnormal prions are detectable but symptoms are not). Moreover, normal homologues of a prion-associated protein exist in the brains of uninfected organisms, further complicating detection. Ivan Roitt, et al., *Immunology* (Mosby-Year Book Europe Limited, 1993), at 15.1.

Current techniques used to detect the presence of prion-related infections rely on gross morphological changes in the brain and immunochemical techniques that are generally applied only after symptoms are manifest. Many of the current detection methods rely on antibody-based assays or affinity chromatography that use brain tissue from dead animals, and in some cases capillary immunoelectrophoresis of blood samples.

Brain-tissue-based assays can lead to late detection and require slaughtering the animal to be tested. Prionic-Check also entails slaughtering an animal to obtain a liquefied-brain tissue sample, which is subjected to an antibody using Western Blot. Although results are obtained in six to seven hours, the test does not account for the six-month lag time between PrP^S accumulation in the brain and the onset of clinical symptoms. Tonsillar biopsy sampling, and blood and cerebrospinal sampling, while accurate, can require surgical intervention and take weeks to obtain results. Electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance NMR, circular dichroism (CD) and other non-amplified structural techniques require large amounts of

sample and expensive equipment that is typically located a substantial distance from the sample source.

Detection methods for conformationally altered proteins associated with the aforementioned disorders such as Alzheimer's disease and CAA are also inadequate in that, like the previously mentioned prion detection techniques, they often require post-mortem tissue sampling,

Accordingly, the need exists for reliable and affordable detection methods for conformationally altered proteins and prions. Such methods should be applicable during the life of the subject at issue in order to obtain rapid diagnoses and facilitate prophylactic or remedial treatments.

SUMMARY OF THE INVENTION

The invention provides reliable, affordable, and safe methods for the detection of conformationally altered proteins and prions associated with a variety of diseases. Methods of the invention can be applied to obtain rapid diagnoses and facilitate prophylactic or remedial treatments. Significantly, the methods of the invention use small amounts of sample and are therefore less invasive and more readily applied than known diagnostic techniques. Further, methods of the invention can be used to analyze samples from a living subject and are not limited to samples obtained post mortem; and may be utilized in a manner that ensures that infectious material is not propagated during testing.

The invention overcomes many of the problems associated with prior art diagnostic techniques by using catalytic propagation to exploit conformational changes in conformationally altered protein or prions associated with a particular disease process, such as transmissible spongiform encephalopathy (TSE). Catalytic propagation may be used to amplify the number of existing conformationally altered protein fragments or prions in a sample and causes detectable aggregates to form as follows:

Upon interaction of a sample containing conformationally altered protein or prions with a conformational probe as defined hereinafter, the probe undergoes a conformational change and adopts the conformation of, and aggregates with, the conformationally altered protein (which may be soluble or insoluble) or prions. The resulting aggregates which exhibit ββ sheet formation, may be readily detected using standard analytical techniques. As a result, the invention facilitates rapid and cost-effective analysis of small sample sizes and is widely applicable to tissues and body fluids from a variety of sources including, but not limited to, the brain.

The invention enables detection of small amounts of disease-associated conformationally altered proteins such as low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntingtin, A-beta peptide, prions, insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, and p53. In a preferred embodiment, methods of the invention use palindromic probes as otherwise described herein, preferably, for example, a palindromic 33_mer probe containing amino acid sequences 126-104 and 109-126 of the $PrP^{(Sc)}$ protein to detect prions in a sample. In a preferred embodiment, the probes are bound at each end to moieties that are optically distinct and detectable upon conformational conversion of the probes to a β-sheet structure.

In one embodiment, the invention provides a method for detecting conformationally altered proteins or prions in a sample comprising:
(a) reacting the sample with one or more α-helix or random coil conformational probes that interact with the ββ-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion to a predominantly ββ-sheet conformation, and (ii) form detectable aggregates with the ββ-sheet conformation insoluble proteins or prions in the sample; and
(b) detecting levels of detectable aggregates,
wherein levels of detectable aggregates correlate to the levels of ββ-sheet conformation insoluble proteins or prions in the sample and the infectiousness of the sample.

The invention also provides kits that use these methods as well as methods of diagnosing whether a subject suffers from, or is predisposed to, a disease associated with conformationally altered proteins or prions.

A kit of the instant invention comprises one or more α-helix or random coil conformational probes that interact with ββ-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion predominantly to ββ-sheet conformation, and (ii) form detectable aggregates with the ββ-sheet conformation insoluble proteins or prions in the sample. The kit may further include moieties that bind to, or are bound to, probe termini and that are optically detectable upon conformational conversion of the probe to a predominantly to ββ-sheet conformation, as well as instructions for using the kit, and solutions for suspending or fixing samples.

A method of diagnosing whether a subject suffers from, or is predisposed to, a disease associated with conformationally altered proteins or prion comprises:
(a) obtaining a sample from the subject;
(b) reacting the sample with one or more α-helix or random coil conformational probes that interact with the ββ-sheet conformation of insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion preferably to a predominantly ββ-sheet conformation, and (ii) form detectable aggregates with the ββ-sheet conformation insoluble proteins or prions in the sample; and
(c) detecting levels of detectable aggregates,
wherein levels of detectable aggregates correlate to the amount of the ββ-sheet conformation insoluble proteins or prions in, and level of infectiousness of, the sample and indicate whether the subject suffers from, or is predisposed to, a disease associated with ββ-sheet conformation insoluble proteins or prions.

These and other aspects of the invention are described further in the following detailed description.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 illustrates the alpha-helical monomer 10 and beta-sheet dimer 12 of a TSE conformer. The normal wild-type (wt) form of prion protein (PrP^{C}) prefers a monomeric state, while the abnormal, disease-causing form (PrP^{Sc}) prefers the multimeric state.

FIGURE 2 illustrates a diagnostic analysis of a sample containing TSE protein comprised of beta-sheets 12.

FIGURE 3 illustrates a circular dichroism graph of a diagnostic analysis that was performed in accordance with the invention and that used a poly-L-lysine 20 micomolar (μM) 52,000 molecular weight (MW) as a peptide -model.

FIGURE 4 illustrates an absorbance graph of a diagnostic analysis that was performed using poly-L-lysine, 70 micromolar (μM) 52,000 molecular weight (MW), as a peptidemodel.

FIGURE 5 illustrates the results from Figure 3, that used a poly-L-lysine, 70 micromolar (μM) 52,000 molecular weight (MW) as a peptide model and the effect of pH and temperature on conformational change.

FIGURE 6 illustrates a spectroscopic analysis that used pyrene as a fluorescent probe in proximal and distal locations in an alpha helical bundle structure that underwent conformational change.

FIGURE 7 illustrates energy changes associated with conformational changes in proteinaceous material or prions.

FIGURE 8 illustrates the alpha-helix and loop structure of PrP.

FIGURE 9 illustrates the predominantly beta-sheet secondary structure of PrP^{Sc}.

FIGURE 10 illustrates a palindromic 33__mer probe used in the methods of the instant invention.

FIGURE 11 illustrates a circular dichroism graph of three distinct common conformational forms that proteins and peptides can assume (source: Woody RW (1996) In Circular Dichroism and the Conformational Analysis of Biomolecules (Fasman, GD ed.) pp. 25-69, Plenum press NY).

FIGURE 12 illustrates a circular dichroism graph of a diagnostic analysis that was performed in aqueous conditions in accordance with the invention and that used a palindromic 33__mer probe and the 14__mer and the 19__mer amino acid sequences which make it up (these three sequences are set forth in FIGURE 10).

FIGURE 13 illustrates a variation of the spectroscopic analysis of Figure 6, in which a spectrofluorometric data of a diagnostic analysis that was performed using a palindromic 33__mer probe (SEQ ID NO: 1, SEE FIGURE 10_) that had pyrene attached to both ends. The spectral scans in the monomer (open) conformation yielded a strikingly fluorescent spectrum that had a maximum emission between 370 and 385 nm, while the excited dimer or excimer state of the pyrene- labeled peptide has an emission max between 475 and 510 nm.

FIGURE 14 illustrates a spectroscopic analysis in which pyrene was used as a fluorophor, the excitation wavelength was around 350 nm, and the observation wavelength was around 365-600 nm. The normal emission of monomer pyrene following excitation (simple fluorescence) was recorded as the maximum wavelength at between about 370-385 nm.

FIGURE 15 illustrates the ratio of excimer formation ((I_D) to monomer formation (I_M) in a diagnostic analysis that used a palindromic 33__mer probe of sequence shown in Fig 10 under various conditions. We expect to see minimum solubility of a protein when the

conditions are near its isoelectric point and that is what we observed where conditions (2) approach the isoelectric point of the 33 __mer peptide- it aggregates with itself since it has dramatically reduced solubility under these conditions as compared to (1) In this example, electrostatic interactions (pI=10) trigger self-association under extremely low concentrations (10 μM) at the isoelectric point of the peptide. The following legend applies to Figure 15.

- **1. pH 6-8, KCl (100-500 mM)**
- **2. pH 10-11, KCl (100-500 mM)**

FIGURE 16 illustrates an associative curve for conformation changes in a diagnostic analysis that used a palindromic 33__mer probe (SEQ ID NO: 1), the 19__mer (SEQ ID NO: 2) and 14 __mer (SEQ ID NO: 3) (See Figure 10) under various conditions to determine the optimal parameters associated with the transformation from coiled to β-sheet.

FIGURE 17 shows the results from the experiment described in Example 6 wherein the fluorescence of a complex of prion protein and 33 __mer probe was measured as a function of time. The complex substantially dissociated over time (1 hour –24 hours).

FIGURE 18 (a)-(c) illustrate fluorescence spectra of target peptide [520 nM] in the presence of infected brain homogenate (1), healthy brain homogenate (2), and peptide alone (3) in TRIS:TFE (1:1) solvent. The data were obtained for 0.01% brain homogenate from hamster (A), sheep (B), and elk (C) (hamster [270 pg/ml], sheep [60 pg/ml], and elk [6 pg/ml]).

FIGURE 19 illustrates a preliminary calibration curve of a fluorescent diagnostic analysis conducted in accordance with the invention. The data illustrated in this figure evidences that the present invention is more than two orders of magnitude more sensitive than the validated tests in use in Europe today without any optimization. *Prion Infectivity:* 1 IU = 3fM = 200,000 PrP

The prion protein concentration was determined using the capillary immunoelectrophoresis method of Dr. Schmerr. See, Schmerr, et al., *J. Chromatogr. A.*, 853 (1-2), 207-214 (August 20, 1999). The sensitivity of the diagnostics with the present invention appears to the left of the green bar, whereas the sensitivity of more conventional diagnostics appears to the right of the green bar. The data are taken from figure 18.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms have the following respective meanings.

"Amyloidogenic diseases" are diseases in which amyloid plaques or amyloid deposits are formed in the body. Amyloid formation is found in a number of disorders, such as diabetes, Alzheimer's Disease (AD), scrapie, Gerstmann-Straussler-Scheinker (GSS) Syndrome, bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD), chronic wasting disease (CWD), and related transmissible spongiform encephalopathies (TSEs).

TSE's are fatal neurodegenerative diseases that include such human disorders as CJD, kuru, fatal familial insomnia, and GSS. Animal forms of TSE include scrapie in sheep, CWD in deer and elk, and bovine spongiform encephalopathy in cattle. These diseases are characterized by the formation and accumulation in the brain of an abnormal proteinase K resistant isoform (PrP-res) of a normal protease-sensitive host-encoded prion protein (PrP-sen). PrP-res is formed from PrP-sen by a post-translational process involving conformational changes that convert the PrP-sen into a PrP-res molecular aggregate having a higher beta.-sheet content. The formation of these macromolecular aggregates of PrP-res is closely associated with TSE-mediated brain pathology in which amyloid deposits of PrP-res are formed in the brain, which eventually becomes "spongiform" (filled with holes).

TSE diseases appear to be transmitted by exposure to an unusual agent, for example by ritual cannibalism in the Foret people of New Guinea, or feeding of animal parts to cattle in bovine spongiform encephalopathy (BSE), iatrogenic CJD has also been caused by administration of human growth hormone derived from cadaveric pituitaries, transplanted dura mater and corneal grafts, as well as exposure of surgeons to affected tissue during neurological procedures.

The presence of a native prion protein (PrP) has been shown to be essential to pathogenesis of TSE. The cellular protein PrP-sen is a sialoglycoprotein encoded by a gene that in humans is located on chromosome 20. The PrP gene is expressed in neural and non-neural tissues, with the highest concentration of its mRNA being in neurons. The translation product of the PrP gene consists of 253 amino acids in humans, 254 in hamsters and mice, 264 amino acids in cows, and 256 amino acids in sheep (all of these sequences are disclosed in U.S. Pat. No. 5,565,186, which describes methods of making transgenic mice that express species specific PrP). In prion protein related encephalopathies, the cellular PrP-sen is converted into the altered PrP-res that is distinguishable from PrP-sen in that PrP-res aggregates (Caughey and Chesebro, 1997, Trends Cell Biol. 7, 56-62); are proteinase K resistant in that only approximately the N-terminal 67 amino acids are removed by proteinase K digestion under conditions in which PrP-sen is completely degraded (Prusiner et al., 1996, Sem. Virol. 7, 159-173); and has an alteration in protein conformation in which the amount of .alpha.-helical conformation for PrP-sen is reduced, and the amount of .beta.-sheet conformation for PrP-res is increased (Pan et al., 1993, Proc. Natl. Acad. Sci. USA 90, 10962-10966).

If PrP-sen is not expressed in the brain tissue of animal recipients of scrapie-infected neurografts, no pathology occurs outside the graft, demonstrating that PrP-res and PrP-sen are both required for the pathology (Brander et al., Nature 379:339-343, 1996). The long latency period between infection and the appearance of disease (months to decades depending on species) has prompted the development of a cell-free in vitro test, in which PrP-res induces the conversion of PrP-sen to PrP-res (Kocisko et al., Nature 370:471474, 1994). See also Prusiner et al., WO 97/16728 published May 9, 1997. These

in vivo and in vitro observations indicate that direct interactions between PrP-res and PrP-sen form PrP-res and promote TSE pathogenesis.

Small synthetic peptides containing certain PrP sequences have previously been shown to spontaneously aggregate to form fibrils with a high degree of .beta.-sheet secondary structure of the type seen in the insoluble deposits in TSE afflicted brains (Gasset et al. , 1992, Proc. Natl. Acad. Sci. USA 89, 10940-10944; Come et al., 1993, Proc. Natl. Acad. Sci. USA 90, 5959-5963; Forloni et al., 1993, Nature 362, 543-546; Hope et al., 1996, Neurodegeneration 5, 1-11). Moreover, other synthetic PrP peptides have been shown to interact with PrP-sen molecules to form an aggregated complex with increased protease-resistance (Kaneko et al., Proc. Natl. Acad. Sci. USA 92, 11160-11164, 1995; Kaneko et al., J. Mol. Biol. 270, 574-586, 1997).

"Conformationally altered proteins" include any protein which has a three dimensional conformation associated with a disease. The conformationally altered protein may cause the disease, may be a factor in a symptom of the disease, or may appear in a sample or *in vivo* as a result of other factors. A conformationally altered protein appears in another conformation which has the same amino acid sequence. These conformationally altered proteins are generally in the form of insoluble proteins exhibiting ββ-sheet formation which are analyzed in the present invention.

The following is a non-limiting list of diseases followed parenthetically by associated insoluble proteins which assemble into two or more different conformations wherein at least one conformation is an example of a conformationally altered protein: Alzheimer's Disease (APP, A.beta. peptide, alpha.1-antichymotrypsin, tau, non-A.beta. component, presenilin 1, presenilin 2 apoE); Prion diseases, Creutzfeld Jakob disease, scrapie and bovine spongiform encephalopathy (PrP^{Sc}); ALS (SOD and neurofilament); Pick's disease (Pick body); Parkinson's disease (alpha.-synuclein in Lewy bodies); Frontotemporal dementia (tau in fibrils); Diabetes Type II (Amylin); Multiple myeloma--plasma cell dyscrasias (IgGL-chain); Familial amyloidotic polyneuropathy (Transthyretin); Medullary carcinoma of thyroid (Procalcitonin); Chronic renal failure

($beta_2$—microglobulin); Congestive heart failure (Atrial natriuretic factor); Senile cardiac and systemic amyloidosis (Transthyretin); Chronic inflammation (Serum amyloid A); Atherosclerosis (ApoA1); Familial amyloidosis (Gelsolin); Huntington's disease (Huntingtin).

An "insoluble protein" includes any protein associated with an amyloidogenic disease, including but not limited to any of the proteins identified in the preceding paragraph. Insoluble proteins generally exhibit ββ-sheet formation in the aggregate.

"PrP protein", "PrP" and like are used interchangeably herein and shall mean both the infectious particle form PrP^{Sc} known to cause diseases (spongiform encephalopathies) in humans and animals and the noninfectious form PrP^{C} which, under appropriate conditions is converted to the infectious PrP^{Sc} form.

The terms "prion", "prion protein", "PrP^{Sc} protein" and the like are used interchangeably herein to refer to the infectious PrP^{Sc} form of a PrP protein. "Prion" is a contraction of the words "protein" and "infection." Particles are comprised largely, if not exclusively, of PrP^{Sc} molecules encoded by a PrP gene. Prions are distinct from bacteria, viruses and viroids. Known prions infect animals and cause scrapie, a transmissible, degenerative disease of the nervous system of sheep and goats, as well as bovine spongiform encephalopathy (BSE), or "mad cow disease", and feline spongiform encephalopathy of cats. Four prion diseases known to affect humans are (1) kuru, (2) Creutzfeldt-Jakob Disease (CJD), (3) Gerstmann-Straussler-Scheinker Disease (GSS), and (4) fatal familial insomnia (FFI). As used herein "prion" includes all forms of prions causing all or any of these diseases or others in any animals used--and in particular in humans and domesticated farm animals.

The term "PrP gene" is used herein to describe genetic material which expresses proteins including known polymorphisms and pathogenic mutations. The term "PrP gene" refers generally to any gene of any species which encodes any form of a prion protein. The PrP gene can be from any animal, and includes all polymorphisms and

mutations thereof, it being recognized that the terms include other such PrP genes that are yet to be discovered. The protein expressed by such a gene can assume either a PrP^{C} (non-disease) or PrP^{Sc} (disease) form.

A "peptidomimetic" is a biomolecule that mimics the activity of another biologically active peptide molecule.

"Protein" refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the .alpha.-carbon of one amino acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the α-carbon of an adjacent amino acid. These peptide bond linkages, and the atoms comprising them (i.e., .alpha.-carbon atoms, carboxyl carbon atoms (and their substituent oxygen atoms), and amino nitrogen atoms (and their substituent hydrogen atoms)) form the "polypeptide backbone" of the protein. In simplest terms, the polypeptide backbone shall be understood to refer the amino nitrogen atoms, .alpha.-carbon atoms, and carboxyl carbon atoms of the protein, although two or more of these atoms (with or without their substituent atoms) may also be represented as a pseudoatom. Indeed, any representation of a polypeptide backbone that can be used in a functional site descriptor as described herein will be understood to be included within the meaning of the term "polypeptide backbone."

The term "protein" is understood to include the terms "polypeptide" and "peptide" (which, at times, may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits (e.g., DNA polymerase III, RNA polymerase II) or other components (for example, an RNA molecule, as occurs in telomerase) will also be understood to be included within the meaning of "protein" as used herein. Similarly, fragments of proteins and polypeptides are also within the scope of the invention and may be referred to herein as "proteins."

"Conformation" or "conformational constraint" refers to the presence of a particular protein conformation, for example, an alpha-helix, parallel and antiparallel beta. strands, leucine zipper, zinc finger, etc. In addition, conformational constraints can include amino acid sequence information without additional structural information. As an example, "--C--X--X--C--" is a conformational constraint indicating that two cysteine residues must be separated by two other amino acid residues, the identities of each of which are irrelevant in the context of this particular constraint. A "conformational change" is a change from one conformation to another.

The exact mechanism by which the sequence of a protein encodes the proper fold is unknown. In order to achieve the native state encoded by the fold, the protein molecule must convert to a unique conformation selected from many alternatives. Functional proteins are typically soluble and can adopt a variety of structures including coils and ordered elements. Ordered elements include the alpha helix predominant in proteins such as myoglobin and hemoglobin. During the human aging process, in some proteins the soluble structure (e.g. alpha helical regions) becomes conformationally altered into beta sheet structures that undergo aggregation associated with loss of function.

There are at least twenty proteins that are associated with human disease when they adopt a conformationally altered state, and some of these have been described previously. Figure 1 illustrates both the alpha-helical monomer 10 and the beta-sheet dimer 12 forms of a TSE conformer. The normal wild-type (wt) form of prion protein (PrP^{C}) prefers a monomeric state, while the abnormal, disease-causing form (PrP^{Sc}) more readily takes on a multimeric state.

Protein structures can be determined by a variety of experimental or computational methods, several of which are described below. Protein structure can be assessed experimentally by any method capable of producing at least low resolution structures. Such methods currently include X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. X-ray crystallography is one method for protein

structural evaluation, and is based on the diffraction of X-ray radiation of a characteristic wavelength by electron clouds surrounding the atomic nuclei in the crystal. X-ray crystallography uses crystals of purified biomolecules (but these frequently include solvent components, co-factors, substrates, or other ligands) to determine near atomic resolution of the atoms making up the particular biomolecule. Techniques for crystal growth are known in the art, and typically vary from biomolecule to biomolecule. Automated crystal growth techniques are also known.

Nuclear magnetic resonance (NMR) currently enables determination of the solution conformation (rather than crystal structure) of biomolecules. Typically only small molecules, for example proteins of less that about 100-150 amino acids, are amenable to these techniques. However, recent advances have lead to the experimental elucidation of the solution structures of larger proteins, using such techniques as isotopic labeling. The advantage of NMR spectroscopy over X-ray crystallography is that the structure is determined in solution, rather than in a crystal lattice, where lattice neighbor interactions can alter the protein structure. The disadvantage of NMR spectroscopy is that the NMR structure is not as detailed or as accurate as a crystal structure. Generally, biomolecule structures determined by NMR spectroscopy are of moderate resolution compared relative to those determined by crystallography.

Other techniques useful in studying biomolecule structure include circular dichroism (CD), fluorescence, and ultraviolet-visible absorbance spectroscopy. See, for example, Physical Biochemistry: Applications to Biochemistry and Molecular Biology, 2.sup.nd ed., W.H. Freeman & Co., New York, N.Y., 1982 for descriptions of these techniques.

"Equivalent" refers to amino acid sequences that are similar in sequence to the amino acid sequence of the protein to be analyzed but have at least one, but fewer than 5, (e.g., 3 or fewer) differences, substitutions, additions, or deletions. Thus, the substitution of one or more amino acid in a given sequence which does not substantially change the

basic function of that amino acid within its use in context, is an equivalent for purposes of describing the present invention.

"Homology", "homologs of", "homologous", or "identity" or "similarity" refers to sequence similarity between two polypeptides, with identity being a more strict comparison. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same amino acid, then the molecules are identical at that position. A degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of amino acids, i.e., structurally related, at positions shared by the amino acid sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences used in the present invention. Related sequences share more than 40% identity, preferably at least about 50% identity, more preferably at least about 70% identity, even more preferably at least about 90% identity, more preferably at least about 99% identity.

The term "percent identical" refers to sequence identity between two amino acid sequences. Identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Various alignment algorithms and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of

Medicine, National Institutes of Health, Bethesda, Md. In one embodiment, the percent identity of two sequences can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid mismatch between the two sequences. Other techniques for determining sequence identity are well-known and described in the art.

The term "interact" as used herein is meant to include detectable interactions (e.g., biochemical interactions) between molecules, such as interaction between protein-protein, protein-nucleic acid, nucleic acid-nucleic acid, and protein-small molecule or nucleic acid-small molecule in nature.

The term "homolog of an insoluble protein" includes all amino acid sequences that are encoded by a homolog of an insoluble protein gene, and all amino acid sequences that are equivalent or homologous to such sequences. Therefore, "homolog of an insoluble protein" includes proteins that are scored as hits in the Pfam family.
To identify the presence of an "insoluble protein" domain in a protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against one of several databases (SwissProt, PIR, for example) using various default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HM_MER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonham_mer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.*(1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.*(1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.*(1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.*(1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

"Test specimen" is a sample of material to be tested. The sample may be prepared from tissue (*e.g.* a portion of ground meat, an amount of tissue obtained by a biopsy procedure) by homogenization in a glass homogenizer. The amount of material should be between about 1mg and 1 gm, preferably between 10 mg and 250 mg, ideally between 20 and 100 mg. The material to be sampled may be suspended in a suitable solvent, preferably phosphate-buffered saline at a pH between 7.0 and 7.8. The solvent may contain a detergent such as (Triton X-100, SDS, or sarkosyl). Homogenization is performed for a number of excursions of the homogenizer, preferably between 10 and 25 strokes; ideally between 15 and 20 strokes. The suspended sample is preferably centrifuged at between 100 and 1,000 g for 5-10 minutes and the supernatant material sampled for analysis. In some samples, it may be preferable to treat the supernatant material with an additional reagent such as phosphotungstic acid according to the procedure described by Safar *et al.*, *Nature Medicine* 4, pp.1157-1165 (1998) and as modified by Wadsworth *et al. The Lancet*, 358, pp.171-180 (2001).

The amount of sample to be tested is based on a determination of the protein content of the supernatant solution as measured by the procedure described by Bradford (1976). Preferably, this corresponds to between 0.5 and 2 mg of protein.

In addition to the procedure described above for tissue material, test samples may be obtained from serum, pharmaceutical formulations that may contain products of animal origin, spinal fluid, saliva, urine or other bodily fluids. Liquid samples may be tested directly or may be subjected to treatment with agents such as phosphotungstic acid as described above.

"Conformational probes" are preferably peptides that have amino acid sequences that are similar to, and more preferably identical to, some of those in the target protein and that also have the potential to undergo conformational alteration to produce ββ-sheet formation when complexed with the target protein (insoluble protein). Such alteration typically leads to a β sheet structure not normally evidenced by the probe. Ideally, a probe has a palindromic structure with two amino acid sequences derived from the target

protein. Preferred α-helix or random coil conformational probes (i.e., probes that exhibit α-helix or random coil conformation in solution) useful in the instant invention include the following :

a palindromic 33__mer comprising amino acid sequences that are identical to amino acids122-104 and 109-122 of the PrP^{SC} protein (SEQ ID NO: 13 and 14) (Swiss-Prot PO4156 (Pfam ID Prion Pf00377 & 03991)

VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine) SEQ ID NO: 29

VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human) SEQ ID NO: 1 ;

a palindromic 33__mer comprising amino acid sequences that are equivalent to amino acids 122-104 and 109-122 of the PrP^{SC} protein SEQ ID NO: 13 and 14) (Swiss-Prot PO4156 (Pfam ID Prion Pf00377 & 03991)

VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine) SEQ ID NO: 29

VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human) SEQ ID NO: 1;

a palindromic 33__mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrP^{SC} protein SEQ ID NO: 13 and 14) (Swiss-Prot PO4156 (Pfam ID Prion Pf00377 & 03991)

VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine) SEQ ID NO: 29

VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human)

a probe comprising amino acid sequences that are identical to amino acids 1-40 of the Abeta peptide (Nref 00111747 (human))

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO: 4);

a probe comprising amino acid sequences that are equivalent to amino acids 1-40 of the Abeta peptide (Nref 00111747 (human))

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO: 4);

a probe comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 1-40 of the Abeta peptide (Nref 00111747 (human))
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO: 4);

a probe comprising amino acid sequences that are identical to amino acids 11-34 of the Abeta peptides (Nref 00111747 (human))
EVHHQKLVFFAEDVGSNKGAIIGL (SEQ ID NO: 5);

a probe comprising amino acid sequences that are identical to amino acids 11-34 of the Abeta peptides (Nref 00111747 (human)) but with residue H13 substituted with R to reduce metal ion interactions and to increase the solubility of the peptide
EVRHQKLVFFAEDVGSNKGAIIGL (SEQ ID NO: 6);

a probe comprising amino acid sequences that are identical to amino acids 25-35 of the Abeta peptides (Nref 00111747 (human))

GSNKGAIIGLM (SEQ ID NO: 7);

a probe that has a helix-loop-helix conformation found in polylysine and an amino acid sequence that is at least 10 amino acid residues in length and is equivalent or homologous to SEQ ID NO:8
KKKKKKKKKKKKKKKKKKKKKKKKKKK (27_mer);

a probe that has a conformation found in polyglutamine and an amino acid sequence that is equivalent or homologous to SEQ ID NO:9
QQQQQQQQQQQQQQQQQQQQQQQQQ;

a probe comprising amino acid sequences that are homologous to amino acids 104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO:10) ;

a probe comprising amino acid sequences that are equivalent to amino acids104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO: 10);

a probe comprising amino acid sequences that are between about 70% to about 90% identical to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO: 10);

a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is homologous to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO: 10);

a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is equivalent to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO: 10);

a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is between about 70% to about 90% identical to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)
KPKTNLKHVAGAAAAGAVV (SEQ ID NO: 10);

a probe comprising amino acid sequences that are identical to amino acids 1-38 of the human islet amyloid polypeptide precursor (amylin) protein (Accession # NP_000406 (human) implicated in human diabetes
MGILKLQVFLIVLSVALNHLKATPIESHQVEKRKCNTA (SEQ ID NO: 11);

a probe comprising amino acid sequences that are identical to at least 10 contiguous amino acid residues within the sequence corresponding to amino acids 1-38 of the human islet amyloid polypeptide precursor (amylin) protein (Accession # NP_000406 (human) implicated in human diabetes

MGILKLQVFLIVLSVALNHLKATPIESHQVEKRKCNTA (SEQ ID NO: 11);

a probe comprising amino acid sequences that are identical to amino acids 1-25 of the human lung surfactant protein (NCBI Accession # AAH32785 (human) implicated in human infant SIDS

MAESHLLQWLLLLLPTLCGPGTAAW (SEQ ID NO: 11);

a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 104 –122 of the human or amino acids 103-121 of the murine PrP^{SC} protein (SEQ ID NO: 13 and 14) (Swiss-Prot PO4156 (Pfam ID Prion Pf00377 & 03991)

Human prion protein
Accession : PO4156 (SEQ ID NO: 13)

```
        10         20         30         40         50         60
         |          |          |          |          |          |
MANLGCWMLV LFVATWSDLG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYP PQGGGGWGQP

        70         80         90        100        110        120
         |          |          |          |          |          |
HGGGWGQPHG GGWGQPHGGG WGQPHGGGWG QGGGTHSQWN KPSKPKTNMK HMAGAAAAGA

       130        140        150        160        170        180
         |          |          |          |          |          |
VVGGLGGYML GSAMSRPIIH FGSDYEDRYY RENMHRYPNQ VYYRPMDEYS NQNNFVHDCV

       190        200        210        220        230        240
         |          |          |          |          |          |
NITIKQHTVT TTTKGENFTE TDVKMMERVV EQMCITQYER ESQAYYQRGS SMVLFSSPPV

       250
         |
ILLISFLIFL IVG
```

#2 Mouse prion protein

Accession : P04925 (SEQ ID NO: 14)

```
         10         20         30         40         50         60
          |          |          |          |          |          |
manlgywlla lfvtmwtdvg lckkrpkpgg wntggsrypg qgspggnryp pqggtwgqph

         70         80         90        100        110        120
          |          |          |          |          |          |
gggwgqphgg swgqphggsw gqphgggwgq gggthnqwnk pskpktnlkh vagaaaagav

        130        140        150        160        170        180
          |          |          |          |          |          |
vgglggymlg samsrpmihf gndwedryyr enmyrypnqv yyrpvdqysn qnnfvhdcvn

        190        200        210        220        230        240
          |          |          |          |          |          |
itikqhtvtt ttkgenftet dvkmmervve qmcvtqyqke sqayydgrrs sstvlfsspp

        250
          |
villisflif livg   ;
```

a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 235-269 (emphasized below) of the human plasma gelsolin (SEQ ID NO: 15) (P06396), Maury, et al. *FEBS Lett.*, 260(1), pp. 85-87 (1990);

```
1 maphrpapal lcalslalca lslpvraata srgasqagap qgrvpearpn smvvehpefl
61 kagkepglqi wrvekfdlvp vptnlygdff tgdayvilkt vqlrngnlqy dlhywlgnec
121 sqdesgaaai ftvqlddyln gravqhrevq gfesatflgy fksglkykkg gvasgfkhvv
181 pnevvvqrlf qvkgrrvvra tevpvswesf nngdcfildl gnnihqwcgs nsnryerlka
241 tqvskgirdn ersgrarvhv seegtepeam lqvlgpkpal pagtedtake daanrklakl
301 ykvsngagtm svslvadenp faqgalksed cfildhgkdg kifvwkgkqa nteerkaalk
361 tasdfitkmd ypkqtqvsvl peggetplfk qffknwrdpd qtdglglsyl sshianverv
421 pfdaatlhts tamaaqhgmd ddgtgqkqiw riegsnkvpv dpatygqfyg gdsyiilyny
481 rhggrqgqii ynwqgaqstq devaasailt aqldeelggt pvqsrvvqgk epahlmslfg
541 gkpmiiykgg tsreggqtap astrlfqvra nsagatrave vlpkagalns ndafvlktps
601 aaylwvgtga seaektgaqe llrvlraqpv qvaegsepdg fwealggkaa yrtsprlkdk
661 kmdahpprlf acsnkigrfv ieevpgelmq edlatddvml ldtwdqvfvw vgkdsqeeek
721 tealtsakry ietdpanrdr rtpitvvkqg feppsfvgwf lgwdddywsv dpldramael
      781 aa
```

YERLKATQVSKGIRDNERSGRARVHVSEEGTEPEAM (SEQ ID NO: 16) ;

a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 27-146 (emphasized below) of the cytastain C protein

sequence depicted below (SEQ ID NO: 17) (P01034), Levy, et al. *J. Exp. Med.*, 169(5), pp. 1771-8 (1989). The amyloid forming version of the peptide is 120 amino acids corresponding to amino acid residues 27-146 below. An appropriate probe is any portion thereof of at least 10 amino acids, numerous probes can be posited accordingly;

```
1   magplrapll llailavala vspaagsspg kpprlvggpm dasveeegvr raldfavgey
61  nkasndmyhs ralqvvrark qivagvnyfl dvelgrttct ktqpnldncp fhdqphlkrk
121 afcsfqiyav pwqgtmtlsk stcqda
```

Palindromic probe of cystatin C protein (from amino acids 39-47 of the above sequence with a four unit proline linker)

EEEVSADMPPPPMDASVEEE ((SEQ ID NO: 18)

a probe comprising amino acid sequences which include at least 10 and up to 23 contiguous glutamine amino acid residues oligo or polyglutamine (from residues 18-40) of the Huntingtin (Huntington's Disease Protein) protein sequence depicted below (SEQ ID NO: 19) (P42858) [gi:1170192]:

```
  1 matleklmka feslksfqqq qqqqqqqqqq qqqqqqqqqq pppppppppp pqlpqpppqa
 61 qpllpqpqpp pppppppppp avaeeplhrp kkelsatkkd rvnhcltice nivaqsvrns
121 pefqkllgia melfllcsdd aesdvrmvad eclnkvikal mdsnlprlql elykeikkng
181 aprslraalw rfaelahlvr pqkcrpylvn llpcltrtsk rpeesvqetl aaavpkimas
241 fgnfandnei kvllkafian lkssptirr taagsavsic qhsrrtqyfy swllnvllgl
301 lvpvedehst llilg.....
```

exemplary probe:
QQQQQQQQQQQQQQQ (SEQ ID NO: 20);

a probe comprising amino acid sequences which include at least 6 contiguous amino acid residues of amino acid residues 12-17 and 15-20 (emphasized below) of the (8-20) domain of the human islet amyloid polypeptide involved in fibrillogenesis, sequence depicted below (SEQ ID NO: 21) NP_000406 [gi:4557655] Scrocchi, et al., *J. Struct. Biol.*, 141(3), pp. 218-27 (2003).

```
1 mgilklqvfl ivlsvalnhl katpieshqv ekrkcntatc atqrlanflv hssnnfgail
```

```
61 sstnvgsnty gkrnavevlk replnylpl
```

Exemplary probes contain the following sequences which are minimal sequences within the sequence 8-20 of the above peptide sequence, which may be used without modification or may be used to form palindromic probes of the present invention:

LANFV (SEQ ID NO: 22);
VFNALPPPPLANFV (palindromic probe) (SEQ ID NO: 23);

FLVHSS (SEQ ID NO: 24);

SSHVLFPPPFLVHSS (palindromic probe) (SEQ ID NO: 25);

a probe comprising amino acid sequences which include at least 5 contiguous amino acid residues of amino acid residues 10-19 (emphasized below) of the peptide fragment of transthyretin, sequence depicted below (SEQ ID NO: 26) AAH20791 [gi:18089145] MacPhee and Dobson, *J. Mol. Biol.*, 297(5), pp. 1203-15 (2000)

```
  1 mashrllllc laglvfvsea gptgtgeskc plmvkvldav rgspainvav hvfrkaaddt
 61 wepfasgkts esgelhgltt eeefvegiyk veidtksywk algispfheh aevvftands
121 gprrytiaal lspysystta vvtnpke  ;
```

a palindromic probe based upon the above referenced sequence (amino acid residues 10-19):

ESVFVLGALPPPPLAGLVFVSE (SEQ ID NO: 27).

Numerous other probes may be readily produced without undue experimentation using standard laboratory techniques and peptide and related chemical syntheses.

The native conformation of the probe is determined by one or more spectroscopic methods such as circular dichroism, Fourier transform infra-red, ultra-violet, nuclear magnetic resonance, or fluorescence, among others. This conformation in a solvent as

described below should correspond to that of an alpha-helix or random coil (in circular dichroism, for example, the nature of the spectrum is indicative of the conformation).

The probe is modified to contain substituents that are detectable by optical means. Such substituents may include tryptophan (an amino acid), pyrene or similar fluorophores, all attached at or near the terminal positions of the peptide probes. Attachment of such fluorophores proceeds according to conventional chemical methods which are well-known in the art, preferably, but not necessarily through covalent attachment of the fluorophore to the probe. Ideally, the substituents have the capability to interact in such a manner as to produce a species known as an excimer. An excimer represents the interaction of two fluorophores that, upon excitation with light of a specific wavelength, emits light at a different wavelength which is also different in magnitude from that emitted by either fluorophore acting alone. Thus, structural alterations of the conformational probe that allow for the formation of such excimers can be detected by a change in optical properties. Such changes can be measured by known fluorimetric techniques, including UV, IR, CD, NMR, or fluorescence, among numerous others, depending upon the fluorophore attached to the probe. The magnitude of these changes is related to the degree to which the probe has undergone the conformational alteration.

In another embodiment, the probe may be substituted with a radioactive material. Ideally, this should be positron emission of a sufficient energy to be detected by machines currently employed for this purpose. Such an entity would preferably contain oxygen-15 (an isotope of oxygen that decays by positron emission) or other radionuclide. In this embodiment, the radiolabeled probe may be injected into a patient and the binding of the probe to the protein target monitored externally.

A probe may comprise a peptide or peptidomimetic of at least five, preferably about ten or more amino acid residues that exhibits a random coil or alpha-helical conformation in solution. A peptide or peptidomimetic probe solvent may be aqueous and have a pH of between about 4 and about 10, preferably between about 5 and about 8, and may have an ionic strength of between about 0.05 and about 0.5 (when typically prepared

with a chloride salt such as sodium chloride or potassium chloride). The solvent may also contain a percentage of a water-miscible organic material such as trifluoroethanol in amounts between about 30 to about 70% by volume, preferably between about 45 to about 60%. The solvent may be prepared with a suitable buffering system such as acetate/acetic acid, Tris, or phosphate.

The sequence of probe amino acids is determined from the nature of the target protein to be analyzed and usually comprises a region of the target that is known to undergo a structural transition from either an alpha-helix or coil to a beta-sheet. This latter structure is associated with the pathogenic form of the target protein. The conformational probe sequence ideally contains two repeats of the target sequence of interest, preferably between about 10 and 25 amino acids in length; more preferably between about 14 and 20 amino acids in length. These are arranged preferably in the probe to form a palindrome as illustrated in Figure 10.

Preferred probes used in methods and kits of the invention have amino acid sequences corresponding to β-sheet regions of the protein to be analyzed. These probes are preferably at least 5 amino acids units in length and can be about 300-400 amino acid units in length (_mer) or more, although, preferably these are about 10 amino acids to about 50 amino acids in length. In certain aspects of the invention, preferred probes which correspond to the β-sheet region are about 15 to about 100 __mer, in others preferred probes may range from about 20 to about 50 __mer. The preferred length of a given probe will be a function of the probes ability to complex and produce ββ-sheet formation with the target protein.

Probes for use in the present invention are readily determined from existing information in sequence databases already in existence or alternatively, may be readily determined experimentally. Thus, the probe will generally correspond to a minimum number of amino acids, preferably at least 10, and more preferably about 10 to 25 amino acids, which correspond to at least a portion of a peptide sequence of a target protein

which undergoing a conformational transition from alpha-helix or random coil to ββ-sheet formation in the insoluble protein.

Noted that within the experimental information which will guide the presentation and synthesis of an appropriate probe, there are some contstraints which can guide the practitioner in making use of the present invention. Because there are only a few kcal difference separating a population in the initial conformation state from a population predominantly in the transformed conformational state (in complex). This transformation is provided by the driving force due either to the Kd of association between the probe molecule and its natural associate to form ββ–sheet complex, or due to changes in the electrostatic interactions between the molecules (for example, by lowering the ionic strength of the solution. If metal ions such as Al are involved, or the binding of another ligand, other electrostatic or steric effects could contribute. The size of the probe peptide can vary, but should be of sufficient length to have "reasonably" well-defined secondary structure under detection conditions and to have sufficient recognitional specificity for the prion selected. The probe peptide should also accommodate single-site mutations in order to be generally applicable to mutated strains, recognizing that these changes and/or heterogeneities affect the thermodynamic stability of the molecule. Moreover, the probe must be non-contagious to the patient population, whether that population is a human patient population, a domesticated animal population or other mammalian population.

Once a peptide sequence is established for a probe (which corresponds to at least a portion of a target protein responsible for β-sheet formation as described above), the peptide sequence may be endcapped (at one, but preferably both ends of the peptide) with a moiety or chemical entity which can facilitate analysis of the peptide probe. Preferably, this moiety is a fluorophore, such as pyrene, but may vary widely, depending upon the analytical technique to be used for analysis. The moiety or chemical entity may be complexed or covalently bonded at or near the amino or carboxy end of the peptide, which is preferably endcapped with a short, hydrophobic peptide sequence. In preferred aspects of the present invention, both the amino and carboxy ends of the probe peptides are endcapped with small hydrophobic peptides ranging in size from about 1 to about 5

amino acids. These may be natural or synthetic, but are preferably natural (i.e., derived from a β-sheet formation region of a target protein. The fluorophore are preferably attached at or near the amino and/or carboxy end of the probe (preferably both) and may be, for example, pyrene, tryptophan, flurescein, rhodamine, among numerous others and is preferably pyrene. It is preferable that the fluorophores form excimers when in the correct geometric orientation.

Conformational probes according to the present invention are preferably palindromic in nature. This refers to the organization of a given conformational probe sequence such that it will contain first and second peptide sequences corresponding to a portion of the target protein responsible for β-sheet formation, but which peptide sequences are presented in a palindromic manner, i.e., from the carboxy end to the amino end (or amino end to carboxy end) in the first peptide sequence, and from the amino end to the carboxy end (or carboxy end to amino end) in the second peptide sequence. The first and second peptide sequence in the palindromic conformational probe do not have to be identical in length, although this may be preferred in certain embodiments, but should be at least roughly equivalent (the two peptide sequences {"arms" of the probe} should not be more than 15, preferably no more than 10 and even more preferably no more than 5 amino acids different in length). Preferably, the first and second peptide sequences within a palindromic probe sequence are separated by a linker comprising between 1 and 5 amino acids, preferably between 1 and 3 amino acids, which preferably contain at least one proline amino acid and more preferably comprise primarily proline amino acids. Figure 10 presents an exemplary palindromic 33_mer conformation probe useful in the present invention.

Preferably, conformational probes according to the present invention contain a hydrophobic amino acid sequence which is preferably derived from the relevant peptide sequence of the target protein (i.e., the peptide sequence responsible for β-sheet formation), and which may vary in length from 1 amino acid to 20 or more amino acids, preferably about 2-10 amino acids in length and appears at or near one of the two ends of the conformation probe. In the case of palindromic conformation probes, these

hydrophobic amino acid sequences appear at the ends of the two peptide arms of the probe. Optionally, the probe also may contain a synthetic hydrophobic amino acid sequence (i.e., not natural to the peptide sequence of the target protein responsible for β-sheet formation) at at least one end of the probe and in the case of palindromic probes, at or near each end of the probe, which may vary in length from as few as one amino acid to 20 or more amino acids, preferably about 3-10 amino acids in length.

By way of example and without limitation, if a desired peptide sequence in a target protein contains the sequence, reading from amino end to carboxy end, QRSTVVURLKAAAV (where AAAV is a hydrophobic amino acid sequence) then the palindrome would contain a first peptide sequence which is VAAAKLRUVVTSRQ and a second peptide sequence which is QRSTVVURLKAAAV (or a close variation to that sequence), with the two sequences separated by a linker comprising from 1 to 5 amino acids, with at least one of those amino acids, and preferably most, if not all, of those amino acids, being proline amino acids. The probe would therefore be:

VAAAKLRUVVTSRQPPPPQRSTVVURLKAAAV SEQ ID NO: 28
(hypothetical palindromic probe)

Preferably, the palindromic probe would contain a hydrophobic amino acid sequence obtained from the relevant sequence of the target protein. Conformational probes according to the present invention may be readily obtained.

The following rules may be used to guide the formation of an appropriate preferred conformational probe according to the present invention. These rules apply generally to conformational probes according to the present invention without limitation, but are more specifically used in context to produce the preferred palindromic conformational probes according to the present invention.

The following rules may be applied to the instant invention to produce preferred conformational peptide probes:

1. Each "arm" of the peptide palindrome should have a minimum of five, and preferably at least 10-12 amino acids and, ideally, not more than about 25 amino acids.
2. The amino acid sequence is selected from a region of a larger protein that is known to undergo a conformational transition from alpha-helix or random coil to beta sheet.
3. One or more of the following additional criteria:

   a) A high proportion of hydrophobic amino acids – generally not less than about 75% (based upon the number of amino acids), ideally 80% or greater

   b) Amino acid repeats of at least 20 and preferably 25 (such as is present in huntingtin)

   c) Clustered charges of opposite sign (as described in Zhang, S., Altman, M. and Rich, A. in Conformational Disease, A Compendium, Solomon, Taraboulos and Katchalski-Katzir, eds. The Center for the Study of Emerging Diseases, 2001.

   d) A linker sequence between each of the peptide arms that has 1 or more amino acids, preferably less than five and that contains one or more proline residues

Test criteria for peptide probe:

1. The conformation of the palindrome peptide probe should be that of an alpha helix or random coil but not a beta sheet.
2. Determination of the conformation of the peptide is ideally accomplished by circular dichroism measurements that can identify solution conformations. These are performed using a CD spectrometer in one or more solvents that can include aqueous buffers and/or organic agents such as trifluoroethanol – see Figure 11.

Applying the general rules obtained above and using readily available methods in the art, one of ordinary skill can produce large numbers of conformational peptide probes having favorable characteristics to be useful in the present invention.

"Circular dichroism" ("CD") is observed when optically active matter absorbs L and R hand circular polarized light slightly differently, as measured by a CD spectropolarimeter. Differences are very small and represent fractions of degrees in ellipticity. Figure 11 depicts an associative CD curve representative of the three distinct common conformational forms that proteins and peptides can assume. CD spectra for distinct types of secondary structure present in peptides and proteins are distinct. Measuring and comparing CD curves of complexed vs uncomplexed protein represents an accurate measuring means of practicing the instant invention.

Unexpectedly, we have determined that under near physiological conditions, the palindrome, 33__mer (SEQ ID NO: 1 or 29), which covalently connects two peptides-the 14__mer (SEQ ID NO: 3 and the 19__mer (SEQ ID NO:2) exhibits a largely coil conformation despite the proximity of two hydrophobic chains resembling the 14__mer structure, as illustrated in Figure 12. The addition of a pyrene at each end of the palindromic 33__mer peptide allows for spectral observation of the conformational change, as illustrated in Figure 13. The spectral scans for pyrene attached to the ends of the 33__mer in the monomer (open) conformation gives a strikingly different fluorescent spectrum, having a maximum emission between 370 and 385 nm, while the excited dimer or excimer state of the pyrene- labeled peptide has an emission max between 475 and 510 nm.

Although it is possible to follow conformational changes by any of the several optical methods described above, a preferred embodiment of the invention utilizes fluorescence spectroscopy since that technique provides sensitivity, rapidity and simplicity of operation. The probe is modified by attachment at both termini of a fluorophore that has specific optical properties. It is preferred that these include the

ability to fluoresce upon irradiation with light of a specific wavelength (defined by the absorption and emission spectra of the chromophore itself). Thus, irradiation with light of a wavelength near that of the absorption maximum and emission of light at a sufficiently higher wavelength so as to be distinguished from the excitation wavelength – this measurement is well known to those versed in the art. Examples of such fluorophores include, but are not limited to, pyrene, tryptophan, fluorescein, rhodamine. It is also preferred that the attached fluorophores have the capacity to form excimers when in the correct geometric orientation.

An "excimer" is an adduct that is not necessarily covalent and that is formed between a molecular entity that has been excited by a photon and an identical unexcited molecular entity. The adduct is transient in nature and exists until it fluoresces by emission of a photon. It is possible to recognize an excimer (or the formation of an excimer) by the production of a new fluorescent band at a wavelength that is longer than that of the usual emission spectrum. An excimer can be distinguished from fluorescence resonance energy transfer since the excitation spectrum is identical to that of the monomer.

The formation of the excimer is dependent on the geometric alignment of the fluorophores and is heavily influenced by the distance between them. In a preferred embodiment, fluorophores are present at each probe terminus and excimer formation between fluorophores is negligible as long as the overall probe conformation is alpha-helix or random coil. This is readily determined by measurement of the fluorescent behavior of the probe in the solvent to be used for analysis in the absence of the target protein to be measured.

Preferred conformational transition following interaction with an analyte target is achieved by measuring fluorescence spectra under conditions where excimer formation can be analyzed. Typically, using pyrene as an exemplary fluorophor, the excitation wavelength would be about 350 nm and the observation wavelength 365-600 nm. The normal emission of monomer pyrene following excitation (simple fluorescence) is

recorded as the maximum wavelength between about 370-385 nm. Representative data is shown in Figure 14.

As shown in Figure 14, the excimer or excited dimer state is recorded at a maximum of between 475 – 510 nm. The formation of the excited dimer state can also be encouraged through the addition of high salt and by conducting measurements at pH approaching the pI of the peptide (e.g., in the illustrated case, a pH of around 10).

Therefore, in a preferred method of the invention, interaction of the probe with the specific protein to be analyzed causes a conformational change in the probe such that excimer formation occurs. This is readily measured by the procedures described herein. Conversion of the probe structure from that exhibited in the absence of analyte (alpha-helix or random coil) to a beta-sheet structure enables fluorophores attached to the probe to form excimers that can be readily identified. Further, the magnitude of excimer formation is directly related to the amount of protein analyte present.

Proteins or prions may be detected in aggregated form or in the presence of other cellular constituents such as lipids, other proteins or carbohydrates. A sample preparation for analysis is preferably homogenized or subjected to a similar disruption of tissue or aggregate structures, and cellular debris is preferably removed by centrifugation. This process is ideally performed in the presence of a buffered salt solution and may utilize one of several detergents such as SDS, Triton X-100, or sarkosyl. Further concentration of the sample may be achieved by treatment with any of several agents; one preferred agent is phosphotungstate, which is employed according to the method of Safar *et al* Nature Medicine 4:1157-1165 (1998).

In a preferred embodiment of the invention, peptide probes are selected in order for addition to an unknown or test sample. The peptide probes are preferably proteins or peptide sequences that have secondary structures of predominately alpha-helix or random coil, but which are preferably, but not necessarily derived from portions of a target peptide responsible for β-sheet formation. In a particularly preferred embodiment, the

peptide probes are peptide fragments consisting of a helix-loop-helix structure found in polylysine. In another particularly preferred embodiment, the peptide probes can be made of a peptide sequence chosen from wild-type (wt) TSE, from a desired species-specific TSE peptide sequence, or even from a selectively mutated TSE sequence that has been mutated in such a manner as to render it destabilized and noninfectious. Additionally, extrinsic fluors such as pyrene can be added or designed into the peptide probe to allow detection of anticipated conformational changes using common fluorescence detection techniques.

Once a peptide probe is selected, it is added to a test sample. Prior to the addition of the peptide probe, however, it is preferred to have the sample subjected to disaggregation techniques commonly known in the art, such as sonication. The disaggregation step allows any potentially aggregated sample material to break apart so that these disaggregated sample materials are free to combine with the newly introduced peptide probe, thereby facilitating the anticipated catalytic propagation.

After the test sample or disaggregated test sample is allowed to interact with the peptide probes, the resulting mixture is then subjected to analytical methods commonly known in the art for the detection of aggregates and to fluorescence measurements in cases where fluorescent peptide probes are used. Unknown or test samples containing any dominant beta-sheet formation characteristic of abnormally folded or disease-causing proteins result in an increase in beta-sheet formation and consequently aggregate formation in the final mixture containing both the test sample and the peptide probes. Conversely, unknown or test samples which lack a predominantly beta-sheet secondary structure will neither catalyze a transition to beta-sheet structure nor will propagate the formation of aggregates.

The initial conformational change can be triggered in the test samples in a number of ways. Without intending to be bound by any theory, the binding of a metal ligand could direct a change in the protein conformation and favor aggregation. The expression or cleavage of different peptide sequences can promote advanced aggregation leading to

fibril and plaque formation. Genetic point mutations can also alter the relative energy levels required of the two distinct conformations, resulting in midpoint shifts in structural transitions. Furthermore, an increase in concentration levels could be sufficient to favor the conformational transition. Regardless of the initial trigger mechanism, however, the disease process in many of the abnormal protein conformations such as in prion-related diseases involves the catalytic propagation of the abnormal conformation, resulting in structural transformation of the previously normal protein.

Optical detection techniques useful in the instant invention include but are not limited to light scattering, or hydrophobicity detection using extrinsic fluors such as 1-anilino-8-napthalene sulfonate (ANS) or Congo Red stain, fluorescence resonance energy transfer (FRET) and quenching of intrinsic tryptophan fluorescence through either conformational change of monomer or binding at an interface in an alpha-beta heterodimer.

Other structural techniques include equilibrium ultracentrifugation or size-exclusion chromotography.

The instant invention uses propagated conformational change to correlate directly levels of abnormal proteins or prions with levels of infectivity. For this reason, it is preferable to utilize the methods of the invention in a manner in which there is no increase in infectious products as a result of the propagation. This can be achieved by placing a "break" in the links between the chain of infection, transmission, and propagation of the abnormal form. Such a "break" must occur at the transitional stage between the dimer and multimer forms of the aggregate. The physical formation of the multimer form can be blocked by simply impeding the step which leads to its formation. This may be achieved by using a probe in which the sequence of interest is attached to a non-relevant peptide, or by a neutral "blocker" segment, with the understanding that probes on linkers or "tethers" are more likely to encounter each other and thus result in amplifying the signal.

The invention is described further in the following examples, which are illustrative and in no way limiting.

EXAMPLE 1

Materials and Methods

A sample may be obtained for testing and diagnosis through use of the instant invention as follows. A sample may be prepared from tissue (*e.g.* a portion of ground meat, or an amount of tissue obtained by a biopsy procedure) by homogenization in a glass homogenizer or by mortar and pestle in the presence of liquid nitrogen. The amount of material should be between about 1mg and 1 gm, preferably between 10 mg and 250 mg, ideally between 20 and 100 mg. The material to be sampled may be suspended in a suitable solvent, preferably phosphate-buffered saline at a pH between 7.0 and 7.8. The addition of Rnase inhibitors is preferred. The solvent may contain a detergent (e.g., Triton X-100, SDS, or sarkosyl). Homogenization is performed for a number of excursions of the homogenizer, preferably between 10 and 25 strokes; ideally between 15 and 20 strokes. The suspended sample is preferably centrifuged at between 100 and 1,000 g for 5-10 minutes and the supernatant material sampled for analysis. In some samples, it may be preferable to treat the supernatant material with an additional reagent such as phosphotungstic acid according to the procedure described by Safar *et al.*, *Nature Medicine*, 4, 1157-1165 (1998) and as modified by Wadsworth, *The Lancet*, 358, 171-180 (2001). Eight prion strains have PrP^{Sc} molecules with different conformations. See, Safar, et al. and Wadsworth, *ibid.* Tissue distribution of protease resistant prion protein in variant Creutzfeldt Jakob disease has been reported using a highly sensitive immunoblotting assay as described in Wadsworth, et al., *ibid.*

The amount of sample to be tested is based on a determination of the protein content of the supernatant solution as measured by the procedure described by Bradford, *Anal. Biochem.* 72:248-254 (1976). A rapid and sensitive method for determining microgram quantities of protein utilizes the principle of protein-dye binding. Preferably, this corresponds to between about 0.5 and 2 mg of protein.

In addition to the procedure described above for tissue material, test samples may be obtained from serum, pharmaceutical formulations that may contain products of animal origin, spinal fluid, saliva, urine or other bodily fluids. Liquid samples may be tested directly or may be subjected to treatment with agents such as phosphotungstic acid as described above.

Illustrative Analysis

A sample containing TSE may be analyzed in accordance with the invention as follows. Referring to Figure 2, the top row of the schematic illustrates an unknown sample of TSE protein represented as containing beta-sheets 12. The beta-sheets are disaggregated by sonication. Labeled peptide probes 14 are added and are allowed to bind to the sample 12. The beta-sheet conformation in sample 12 induces the peptide probes to conform to beta-sheet conformation 16. Beta-sheet propagation among the peptide probes 14 forms aggregates 18. The resulting transition to a predominately beta-sheet form and amplified aggregate formation is detected by techniques such as light scattering and circular dichroism (CD). In a particularly preferred embodiment, the peptide probe is fluorescently labeled and fluorescence detection is used.

The bottom row of Figure 2 shows an alternative example in which the unknown sample of TSE protein is represented in its normal alpha-helical form 10. For consistency, the sample is subjected to the same disaggregation process described above. Upon addition of the labeled peptide probes 14, neither a transition to beta-sheet form nor binding to the unknown samples occurs. As a result, there is no aggregate fluorescence signal in the case of a labeled peptide probe and there is no detection of aggregate formation by other analytical tools. Based on this schematic, unknown samples can be tested for the presence or absence of such abnormal protein conformations or sequences.

EXAMPLE 2

Poly-lysine was used as a model peptide. Experiments were performed using model systems to illustrate the conformational changes involved in the transition from a

predominately alpha-helix to a beta-rich form. The model system chosen used non-neurotoxic polyamino acid polylysine.. The polyamino acid was chosen because of availability and safety; and normally evidences random coil conformation at pH values between 5 and 9.

Figure 3 depicts a CD graph of an experiment in which poly-L-lysine 20 micro Molar (μμM) 52,000 molecular weight (MW) was used as a peptide model.

As also illustrated in Figure 3:

Sample 24, which was maintained at pH 7, 25°C, exhibited a minimum at approximately 205 nanometers (nm), indicating a random coil structure;

Sample 26 which was maintained at pH 11 (near the isoelectric point), at 50° C, resulted in a minimum at approximately 216 nanometers (nm) indicating a β-sheet structure (see Figure 11 for exemplary CD spectra of protein conformations);

Sample 28, which was a 1:1 combination of samples maintained at pH7, 25°C and at pH11, 50° C , resulted in a minimum at approximately 216 namometers (nm) indicating β-sheet structure;

Sample 30, which was a 1:1 combination of samples maintained at pH 7, 50° C and at pH 11, 50° C, resulted in a minimum at approximately 216 namometers (nm), indicating β-sheet structure.

EXAMPLE 3

Figure 4 illustrates general CD results of experiments that were conducted: (1) using poly-L-lysine; and (2) at varying temperatures and pH, to observe the effect of random coil to beta-sheet conformational changes under varying environmental conditions. The results indicate that both temperature and pH play an important role in the transition. The results also indicate that the addition of a relatively small amount of β-sheet peptide to a random coil sample can result in a shift towards a β-rich conformation and that such changes can be accelerated depending on the temperature and pH environment of the samples.

More specifically, Figure 4 illustrates an absorbance graph generated using a poly-L-lysine of 52,000 molecular weight (MW) at 70 micromolar (μM) as a peptide probe in accordance with the experimental technique described in Examples 1-3. Figure 4 illustrates that:

Sample 32 (pH 11, 25°C) evidenced a plateau at approximately 0.12, indicating a predominantly α-helical structure;

Sample 34 (maintained at pH 7, 50°C) evidenced a plateau at approximately 0.22, which indicated a predominantly random coil structure;

Sample 36 (a 10:1 combination of samples maintained at pH 7, 50°C and at pH 11, 50°C) resulted in a steeper incline from approximately 0.22 to 0.33, indicating an accelerated transition from random coil to β-sheet structure;

Sample 38 (a 10:1 combination of samples maintained at pH 7, 25°C and at pH 11, 50°C) resulted in a gradual incline from approximately 0.22 to 0.26, indicating a transition from random coil to β-sheet structure.

The observations based on all of the experiments described above show that the addition of a relatively small amount of β-sheet peptide to random coil sample can result in a shift towards a beta-rich conformation and that such changes can be accelerated depending on the temperature and pH environment of the samples.

EXAMPLE 4

The experiment that led to the results illustrated in Figure 15 involved use of the 33 __mer target peptide (SEQ ID NO:1 and 29)

VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine)

VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human)

alone, and probing peptide association through the observation of excimer formation.

The 33 __mer target peptide (SEQ ID NO:1 or 29) used was a murine amino acid sequence which differed from a corresponding human sequence in the substitution of methionine for valine and leucine at positions _M11V_, M14L__, _M20L_, and M23V__, as illustrated in Figure 10B. We compared the results we observed using CD (in which peptides were unlabeled) and spectrofluorometric studies (using pyrene-labeled peptides). No homogenate was used. The experiment that lead to the results illustrated in Figure 15 was a detailed study undertaken to understand what triggered the 33 __mer target peptide (SEQ ID NO:1 or 29) to conformationally change from predominately monomeric to dimeric (excimeric) and become aggregated. Conditions were found that encouraged 33__mer labeled-peptide association in the μM-range.

Conditions that screened the electrostatic interactions of the 33__mer target peptide and thereby minimized its solubility (pI=10) triggered self-association of the peptide under extremely low concentrations (10 μM). This self association is evident in the formation of dimers or excimers and the concomitant far red shift in fluorescence by virtue of the pyrene fluorophor on the ends of the peptides. As an example, Curve 1 of Figure 15 represents the conditions of pH 6-8, KCl (100-500 μM) where the predominant peptide conformer is monomeric; while Curve 2 of figure 15 represents the conditions of pH 10-11, KCl (100-500 μM), where at very low concentrations of peptide, we observed strong excimer formation (aggregation of the monomers).

EXAMPLE 5

The experiment that led to the results illustrated in Figure 16 involved use of various individual peptides, and the 33__mer probe (comprising 19 _mer and 14_mer) target peptide (SEQ ID NO:1, 29, 2 or 3)
VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine)
VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human) The assay conditions were changed to observe the effect on conformation as monitored by CD. The goal was to determine what thermodynamic conditions result in one step transition from monomeric

random coil to aggregated β sheet and avoid the associative 'X' state that is probably micelle formation of the peptides.

In the experiment that lead to the results illustrated in Figure 16, a specific λ (205 nm) wavelength was used to monitor peptide association by CD to obtain detailed conformational information over a range of solvent conditions and across a range of peptide concentrations (peptide concentrations are presented in log scale and also refer to the standard diagram for CD – Figure11).

The associative curve (θ_{205}) recovered for the target peptides showed two conformational transitions at the 50 μM and 3 mM range, respectively, moving from a coil through to 'X' state and to β-sheet.

Referring to Figure 16, for solvent conditions above 50% (far left dashed line), the 33_mer target peptide (SEQ ID NO:1 and 29)

VVAGAAAAGAVHKLNTKPKLKHVAGAAAAGAVV (murine)

VVAGAAAAGAMHKMNTKPKMKHMAGAAAAGAVV (human)

transitioned from the coil state to a β-sheet state at 3 μM, while the component 19 _mer or 14 _mer were able to transition, but at nearly 10-fold higher peptide concentration (middle line). Under aqueous conditions, (thick line) none of the peptides were able to self associate into a β sheet structure.

The 33_mer palindromic peptide target peptide (SEQ ID NO:1 and 29) exhibited unique properties at very low concentrations (ie. 1 μM) under 50% solvent (acetonitrile or trifluoroethanol) conditions in that it avoided the "dead-end" associative state (as exhibited by the plateauing effect under aqueous conditions).

FIGURE 16 shows that a variation in solvent and temperature does not significantly affect the associative behavior of target peptides and that all of the peptides follow the same curve, indicating that sequence specificity is not an important feature in this kind of molecular assembling.

EXAMPLE 6

The experiment that led to the results illustrated in Figure 17 was conducted as follows.

One gram of scrapie infected (strain 293) hamster brain material was homogenized in liquid nitrogen in sterile phosphate buffered saline. Ten-fold serial dilutions were made into sterile PBS. The concentration of protease resistant prion protein (PrP^{Sc}) in the brain homogenates was determined by capillary electrophoresis antibody-capture. Brain homogenate equivalent to 10 ng of protease resistant prion protein (PrP^{Sc}) was mixed with 1.5 μM of 33 _mer target peptide in 50% TFE (trifluoroethanol) and incubated for 1 hour at room temperature prior to excitation at 350 nm in a dual chronometer spectrofluorometer and emission from 350 to 600 nm recorded, the excitation and emission scan was repeated at 5 hours and 24 hours. The 33_mer peptide alone was used as a control .

Addition of the infectious prion protein led to the significant increase in the fluorescence of the 33-_mer target peptide, which was found to be in near β-sheet conformation by CD data under conditions of 50% Tris:50% TFE. This increase of fluorescence indicated the formation of 33_meraggregates. The 33_mer aggregates were found to be unstable and dissociated irreversibly with time.

Following the emission of fluorescence for the complex versus the peptide over time illustrated that the complex dissociated with time, while the peptide fluorescence remained stable monitoring at two different wavelengths, 377 nm (triangle) and 475 nm (square).

EXAMPLE 7

The experiment that led to the results illustrated in FIGURE 18 was conducted as follows.

One gram of scrapie infected and healthy hamster brain, sheep brain and elk brain were homogenized in liquid nitrogen in sterile phosphate buffered saline. Ten-fold serial dilutions were made into sterile PBS. The concentration of protease resistant prion protein (PrP^{Sc}) in the brain homogenates was determined by capillary electrophoresis antibody-capture. Brain homogenates, infected and healthy, were mixed with 0.52 μM of 33 _mer target peptide in 50% TFE (trifluoroethanol):50% TRIS and incubated for 1 hour at room temperature prior to excitation at 350 nm in a dual chromometer spectrofluorometer and emission at 350 to 600 nm recorded. The 33_mer peptide alone in 50%TFE:50% TRIS was used as an additional control.

Fluorescence spectra of the target peptide [520 nM] in the presence of infected brain homogenate(graph line 1-), healthy brain homogenate (graph line 2-), and peptide alone (graph line 3-) in TRIS:TFE (1:1) solvent are shown in FIGURE 18. The data are for 0.01% brain homogenate from hamster (panel A), sheep (panel B), and elk (panel C). hamster [270 pg/ml], sheep [60 pg/ml], and elk [6 pg/ml].